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RAW SEQUENCE LISTING  
PATENT APPLICATION: US/10/017,145

DATE: 01/10/2002  
TIME: 15:51:00

Input Set : A:\ES.txt  
Output Set: N:\CRF3\01102002\J017145.raw

ENTERED

3 <110> APPLICANT: Brookhaven Science Associates  
4 Shanklin, John  
6 <120> TITLE OF INVENTION: Mutant Fatty Acid Desaturase and Methods for Directed  
Mutagenesis  
8 <130> FILE REFERENCE: CIP of 09/328,550 filed June 9, 1999; which was a CIP of  
09/233,856 filed  
W--> 9 January 19, 1999  
C--> 11 <140> CURRENT APPLICATION NUMBER: US/10/017,145  
C--> 11 <141> CURRENT FILING DATE: 2001-12-14  
11 <150> PRIOR APPLICATION NUMBER: 09/328,550  
12 <151> PRIOR FILING DATE: 1999-06-09  
14 <160> NUMBER OF SEQ ID NOS: 13  
16 <170> SOFTWARE: PatentIn version 3.1  
18 <210> SEQ ID NO: 1  
19 <211> LENGTH: 363  
20 <212> TYPE: PRT  
21 <213> ORGANISM: Ricinus communis  
23 <220> FEATURE:  
24 <221> NAME/KEY: misc\_feature  
25 <223> OTHER INFORMATION: ricinus communis delta 9 18:0 Acyl ACP Desaturase  
28 <400> SEQUENCE: 1  
30 Ala Ser Thr Leu Lys Ser Gly Ser Lys Glu Val Glu Asn Leu Lys Lys  
31 1 5 10 15  
34 Pro Phe Met Pro Pro Arg Glu Val His Val Gln Val Thr His Ser Met  
35 20 25 30  
38 Pro Pro Gln Lys Ile Glu Ile Phe Lys Ser Leu Asp Asn Trp Ala Glu  
39 35 40 45  
42 Glu Asn Ile Leu Val His Leu Lys Pro Val Glu Lys Cys Trp Gln Pro  
43 50 55 60  
46 Gln Asp Phe Leu Pro Asp Pro Ala Ser Asp Gly Phe Asp Glu Gln Val  
47 65 70 75 80  
50 Arg Glu Leu Arg Glu Arg Ala Lys Glu Ile Pro Asp Asp Tyr Phe Val  
51 85 90 95  
54 Val Leu Val Gly Asp Met Ile Thr Glu Glu Ala Leu Pro Thr Tyr Gln  
55 100 105 110  
58 Thr Met Leu Asn Thr Leu Asp Gly Val Arg Asp Glu Thr Gly Ala Ser  
59 115 120 125  
62 Pro Thr Ser Trp Ala Ile Trp Thr Arg Ala Trp Thr Ala Glu Glu Asn  
63 130 135 140  
66 Arg His Gly Asp Leu Leu Asn Lys Tyr Leu Tyr Leu Ser Gly Arg Val  
67 145 150 155 160  
70 Asp Met Arg Gln Ile Glu Lys Thr Ile Gln Tyr Leu Ile Gly Ser Gly  
71 165 170 175  
74 Met Asp Pro Arg Thr Glu Asn Ser Pro Tyr Leu Gly Phe Ile Tyr Thr  
75 180 185 190  
78 Ser Phe Gln Glu Arg Ala Thr Phe Ile Ser His Gly Asn Thr Ala Arg  
79 195 200 205  
82 Gln Ala Lys Glu His Gly Asp Ile Lys Leu Ala Gln Ile Cys Gly Thr

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86 Ile Ala Ala Asp Glu Lys Arg His Glu Thr Ala Tyr Thr Lys Ile Val  
 87 225 230 235 240  
 90 Glu Lys Leu Phe Glu Ile Asp Pro Asp Gly Thr Val Leu Ala Phe Ala  
 91 245 250 255  
 94 Asp Met Met Arg Lys Lys Ile Ser Met Pro Ala His Leu Met Tyr Asp  
 95 260 265 270  
 98 Gly Arg Asp Asp Asn Leu Phe Asp His Phe Ser Ala Val Ala Gln Arg  
 99 275 280 285  
 102 Leu Gly Val Tyr Thr Ala Lys Asp Tyr Ala Asp Ile Leu Glu Phe Leu  
 103 290 295 300  
 106 Val Gly Arg Trp Lys Val Asp Lys Leu Thr Gly Leu Ser Ala Glu Gly  
 107 305 310 315 320  
 110 Gln Lys Ala Gln Asp Tyr Val Cys Arg Leu Pro Pro Arg Ile Arg Arg  
 111 325 330 335  
 114 Leu Glu Glu Arg Ala Gln Gly Arg Ala Lys Glu Ala Pro Thr Met Pro  
 115 340 345 350  
 118 Phe Ser Trp Ile Phe Asp Arg Gln Val Lys Leu  
 119 355 360  
 122 <210> SEQ ID NO: 2  
 123 <211> LENGTH: 1092  
 124 <212> TYPE: DNA  
 125 <213> ORGANISM: Ricinus communis  
 127 <220> FEATURE:  
 128 <221> NAME/KEY: misc\_feature  
 129 <223> OTHER INFORMATION: residues 138 to 1239 of open reading frame  
 132 <400> SEQUENCE: 2  
 133 gcctctaccc tcaagtctgg ttctaaggaa gttgagaatc tcaagaagcc tttcatgcct 60  
 135 cctcgggagg tacatgttca gtttacccat tctatgccac cccaaaagat tgagatcttt 120  
 137 aaatccctag acaattgggc tgaggagaac attctggttc atctgaagcc agttgagaaa 180  
 139 tggggcaac cgcaggattt tttgccagat cccgcctctg atggatttga tgagcaagtc 240  
 141 agggaaactca gggagagagc aaaggagatt cctgatgatt atttgttgtt tttgggttgg 300  
 143 gacatgataa cggaaaagc cttcccaact tatcaaaca tgctgaatac cttggatgg 360  
 145 gttcgggatg aaacaggtgc aagtctact tctttggcaa tttggacaag ggcatggact 420  
 147 gccggaaagaga atagacatgg tgaccccttc aataagtatc tctacccatc tggacgagtg 480  
 149 gacatgagggc aaatttgagaa gacaattcaa tatttgattt gttcaggaat ggatccacgg 540  
 151 acagaaaaca gtccataccct tgggttcatac tatacatcat tccaggaaag ggcaaccttc 600  
 153 atttctcatg ggaacactgc cggacaagcc aaagagcatg gagacataaa gttggctcaa 660  
 155 atatgtggta caattgctgc agatgagaag cggccatgaga cagccctacac aaagatagt 720  
 157 gaaaaactct ttgagattga tcctgtatgaa actgtttgg ctttgtctga tatgtatgaga 780  
 159 aagaaaaattt ctatgcctgc acacttgatg tatgtatggcc gagatgataa tcttttgac 840  
 161 cactttcag ctgttgcgc gctgttgcgtt gtcgttgcgtt gaaaggatca tgcgttgcgtt 900  
 163 ttggagttct tgggtggcag atggaagggtt gataaaactaa cggcccttcc agctgggg 960  
 165 caaaaaggctc aggactatgt ttgtcggtt cctccaagaa ttggatgtt ggaaggagaga 1020  
 167 gctcaaggaa gggcaaaaggaa agcaccaccat atgccttca gctggatgtt cgataggcaa 1080  
 169 gtgaagctgtt ag 1092  
 172 <210> SEQ ID NO: 3  
 173 <211> LENGTH: 34  
 174 <212> TYPE: DNA  
 C--> 175 <213> ORGANISM: Artificial

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177 &lt;220&gt; FEATURE:

178 &lt;221&gt; NAME/KEY: misc\_feature

179 &lt;223&gt; OTHER INFORMATION: PCR primer; sequence flanking unique XbaI site at the 5' end

of t

180 he open reading frame

183 &lt;400&gt; SEQUENCE: 3

184 gtgagcggat aacaatttca cacagtctag aaat

34

187 &lt;210&gt; SEQ ID NO: 4

188 &lt;211&gt; LENGTH: 72

189 &lt;212&gt; TYPE: DNA

C--&gt; 190 &lt;213&gt; ORGANISM: Artificial

192 &lt;220&gt; FEATURE:

193 &lt;221&gt; NAME/KEY: misc\_feature

194 &lt;222&gt; LOCATION: (56)..(57)

195 &lt;223&gt; OTHER INFORMATION: PCR primer is a degenerate oligonucleotide in which "n" indicates

196 the presence of either C, A, T or G at that nucleotide position

199 &lt;400&gt; SEQUENCE: 4

W/ 200 ccaaattgcc caagacgtcg gacttgcacc tgtttcatcc cgaactccat ccaamnnatt 60  
202 cagcattgtt tg 72

205 &lt;210&gt; SEQ ID NO: 5

206 &lt;211&gt; LENGTH: 31

207 &lt;212&gt; TYPE: DNA

C--&gt; 208 &lt;213&gt; ORGANISM: Artificial

210 &lt;220&gt; FEATURE:

211 &lt;221&gt; NAME/KEY: misc\_feature

212 &lt;223&gt; OTHER INFORMATION: PCR primer

215 &lt;400&gt; SEQUENCE: 5

216 gaaaacaggtg caagtcggac gtcttggca a 31

219 &lt;210&gt; SEQ ID NO: 6

220 &lt;211&gt; LENGTH: 26

221 &lt;212&gt; TYPE: DNA

C--&gt; 222 &lt;213&gt; ORGANISM: Artificial

224 &lt;220&gt; FEATURE:

225 &lt;221&gt; NAME/KEY: misc\_feature

226 &lt;223&gt; OTHER INFORMATION: PCR primer

229 &lt;400&gt; SEQUENCE: 6

230 gttttctgtc cgcggatcca ttcctg 26

233 &lt;210&gt; SEQ ID NO: 7

234 &lt;211&gt; LENGTH: 34

235 &lt;212&gt; TYPE: DNA

C--&gt; 236 &lt;213&gt; ORGANISM: Artificial

238 &lt;220&gt; FEATURE:

239 &lt;221&gt; NAME/KEY: misc\_feature

240 &lt;223&gt; OTHER INFORMATION: PCR primer

243 &lt;400&gt; SEQUENCE: 7

244 gtgagcggat aacaatttca cacagtctag aaat

34

247 &lt;210&gt; SEQ ID NO: 8

248 &lt;211&gt; LENGTH: 30

249 &lt;212&gt; TYPE: DNA

C--&gt; 250 &lt;213&gt; ORGANISM: Artificial

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252 <220> FEATURE:  
 253 <221> NAME/KEY: misc\_feature  
 254 <223> OTHER INFORMATION: PCR primer  
 257 <400> SEQUENCE: 8  
 258 cacgaggccc tttcggtttc aagaattctc 30  
 261 <210> SEQ ID NO: 9  
 262 <211> LENGTH: 28  
 263 <212> TYPE: DNA  
**C--> 264 <213> ORGANISM: Artificial**  
 266 <220> FEATURE:  
 267 <221> NAME/KEY: misc\_feature  
 268 <223> OTHER INFORMATION: PCR primer  
 271 <400> SEQUENCE: 9  
 272 ttgataagtg ggaaggggctt cttccgtt 28  
 275 <210> SEQ ID NO: 10  
 276 <211> LENGTH: 66  
 277 <212> TYPE: DNA  
**C--> 278 <213> ORGANISM: Artificial**  
 280 <220> FEATURE:  
 281 <221> NAME/KEY: misc\_feature  
 282 <222> LOCATION: (41)..(43)  
 283 <223> OTHER INFORMATION: PCR primer is a degenerate oligonucleotide in which "n" indicates  
 284 the presence of either C, A, T or G and in which "k" indicates t  
 285 he presence of either T or G.  
 288 <220> FEATURE:  
 289 <221> NAME/KEY: misc\_feature  
 290 <222> LOCATION: (32)..(34)  
 291 <223> OTHER INFORMATION: PCR primer is degenerate oligonucleotide in which "n" indicates th  
 292 e presence of either C, A T, or G at that nucleotide position and  
 293 in which "k" indicates either T or G  
 296 <220> FEATURE:  
 297 <221> NAME/KEY: misc\_feature  
 298 <222> LOCATION: (44)..(46)  
 299 <223> OTHER INFORMATION: PCR primer is a degenerate oligonucleotide in which "n" indicates  
 300 the presence of either C, A, T, or G at that nucleotide position  
 301 and in which "k" indicates the presence of either T or G.  
**W-4> 304 <400> SEQUENCE: 10**  
 305 aacggaagaa gccctccca cttatcaaac annkctgaat nnknnkgtg gagttcggga 60  
 307 tgaaac 66  
 310 <210> SEQ ID NO: 11  
 311 <211> LENGTH: 26  
 312 <212> TYPE: DNA  
**C--> 313 <213> ORGANISM: Artificial**  
 315 <220> FEATURE:  
 316 <221> NAME/KEY: misc\_feature  
 317 <223> OTHER INFORMATION: PCR primer  
 320 <400> SEQUENCE: 11  
 321 tccattcctg aaccaatcaa atattg 26  
 324 <210> SEQ ID NO: 12

## RAW SEQUENCE LISTING

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Input Set : A:\ES.txt

Output Set: N:\CRF3\01102002\J017145.raw

325 &lt;211&gt; LENGTH: 70

326 &lt;212&gt; TYPE: DNA

C--&gt; 327 &lt;213&gt; ORGANISM: Artificial

329 &lt;220&gt; FEATURE:

330 &lt;221&gt; NAME/KEY: misc\_feature

331 &lt;222&gt; LOCATION: (22)..(24)

332 &lt;223&gt; OTHER INFORMATION: PCR primer in a degenerate oligonucleotide in which "n" indicates

333 the presence of either C, A, T or G at that nucleotide position  
334 and in which "k" indicates the presence of either T or G at that  
335 nucleotide position.

338 &lt;220&gt; FEATURE:

339 &lt;221&gt; NAME/KEY: misc\_feature

340 &lt;222&gt; LOCATION: (28)..(30)

341 &lt;223&gt; OTHER INFORMATION: PCR primer in a degenerate oligonucleotide in which "n" indicates

342 the presence of either C, A, T or G at that nucleotide position  
343 and in which "k" indicates the presence of either T or G at that  
344 nucleotide position.

347 &lt;220&gt; FEATURE:

348 &lt;221&gt; NAME/KEY: misc\_feature

349 &lt;222&gt; LOCATION: (49)..(51)

350 &lt;223&gt; OTHER INFORMATION: PCR primer in a degenerate oligonucleotide in which "n" indicates

351 the presence of either C, A, T or G at that nucleotide position  
352 and in which "k" indicates the presence of either T or G at that  
353 nucleotide position.

356 &lt;400&gt; SEQUENCE: 12

W~~Y~~ 357 ttgattgggtt caggaatgga tnnkcggnk gaaaacagtc catacctnn kttcatctat 60  
359 acatcattcc 70

362 &lt;210&gt; SEQ ID NO: 13

363 &lt;211&gt; LENGTH: 30

364 &lt;212&gt; TYPE: DNA

C--&gt; 365 &lt;213&gt; ORGANISM: Artificial

367 &lt;220&gt; FEATURE:

368 &lt;221&gt; NAME/KEY: misc\_feature

369 &lt;223&gt; OTHER INFORMATION: PCR primer

372 &lt;400&gt; SEQUENCE: 13

373 gcaaaagcca aaacggtacc atcaggatca 30

## VERIFICATION SUMMARY

PATENT APPLICATION: US/10/017,145

DATE: 01/10/2002

TIME: 15:51:01

Input Set : A:\ES.txt

Output Set: N:\CRF3\01102002\J017145.raw

L:9 M:259 W: Allowed number of lines exceeded, <130> FILE REFERENCE:  
L:11 M:270 C: Current Application Number differs, Replaced Current Application No  
L:11 M:271 C: Current Filing Date differs, Replaced Current Filing Date  
L:175 M:220 C: Keyword misspelled or invalid format, <213> ORGANISM for SEQ ID#:3  
L:190 M:220 C: Keyword misspelled or invalid format, <213> ORGANISM for SEQ ID#:4  
L:200 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:4  
L:208 M:220 C: Keyword misspelled or invalid format, <213> ORGANISM for SEQ ID#:5  
L:222 M:220 C: Keyword misspelled or invalid format, <213> ORGANISM for SEQ ID#:6  
L:236 M:220 C: Keyword misspelled or invalid format, <213> ORGANISM for SEQ ID#:7  
L:250 M:220 C: Keyword misspelled or invalid format, <213> ORGANISM for SEQ ID#:8  
L:264 M:220 C: Keyword misspelled or invalid format, <213> ORGANISM for SEQ ID#:9  
L:278 M:220 C: Keyword misspelled or invalid format, <213> ORGANISM for SEQ ID#:10  
L:305 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:10  
L:313 M:220 C: Keyword misspelled or invalid format, <213> ORGANISM for SEQ ID#:11  
L:327 M:220 C: Keyword misspelled or invalid format, <213> ORGANISM for SEQ ID#:12  
L:357 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:12  
L:365 M:220 C: Keyword misspelled or invalid format, <213> ORGANISM for SEQ ID#:13